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METHODS FOR THE ANALYSIS OF TRANSIENT ABSORBANCE DATA

(Technical Report)

Prepared for publication by

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Methods for the analysis of transient absorbance data (Technical Report)

Abstract: Procedures for the generation and collection of transient UV-visible absorbance data are briefly reviewed. Problems associated with signal generation (scattered light, inhomogeneous distribution of transients, instability of pulsed light sources), signal detection (averaging, filtering), and signal analysis in kinetic and spectrographic flash photolysis are addressed. Methodology for the fitting of model functions to absorbance data that depend on up to three variables (time, wavelength and, e.g., temperature) is discussed.

I. INTRODUCTION

Since its invention in 1949 [1], flash photolysis has remained one of the most important tools to detect short-lived intermediates and, thus, to elucidate elementary reaction steps. The time resolution has increased dramatically from milliseconds to femtoseconds. Most researchers construct their own instruments from commercial components and design their own software for instrument control, data collection, and data analysis. The purpose of this document is to increase the awareness about available methods, their pitfalls ("*beware of artifacts!*") and their advantages, for the generation, collection, and analysis of transient absorbance data. Instrument design is considered only in passing, inasmuch as it pertains to data collection and analysis. For more technical detail, the reader is referred to several reviews. An excellent article by Porter and West [2], written in 1973, describes the basic principles that are still employed today. The same volume of "Techniques of Chemistry" [2] contains a series of reviews on other fast reaction techniques including stopped flow, pulse radiolysis, temperature and pressure jump, to which most considerations of the present article apply directly or by analogy. Since those days we have seen tremendous developments in the production of short laser pulses, in the time resolution of detectors and digitizers, and in the speed and memory capacities of computers. More recent reviews can be found in two handbooks [3, 4] and several monographs [5]. Many descriptions of individual instruments have been reported [6]. Experimental techniques used for diffuse reflectance flash photolysis of heterogeneous and opaque systems and special problems associated with that technique have been discussed elsewhere [3b, 4b, 7].

An article entitled "Recommended Methods for Fluorescence Decay Analysis" was published in this Journal [8]. The methods for the analysis of time-resolved optical spectra are basically the same for absorption and emission [9]. However, the methods for data collection and many practical problems of data analysis differ substantially. Instrument stability is usually excellent for fluorescence decay measurements and permits prolonged accumulation as well as a reliable deconvolution of the instrument response function. Data averaging traditionally played a less important role in flash photolysis but is becoming indispensable with ultrashort pulsed lasers; repetition rates used for averaging have reached several kHz. Much faster repetition rates, such as the 100 MHz commonly used for fluorescence measurements, cannot be achieved because the measurement of absorbance requires intense probe pulses. Prolonged accumulation may be impeded by non-random variations such as the gradual changes of laser pulse intensities, baseline drifts, sample degradation, etc.

Fully computer-controlled, digital data collection is not merely a convenience. In practice, it is prerequisite to obtain a sufficient amount of data for a clear-cut discrimination between all but the simplest reaction models encountered in kinetics. Conversely, proper data analysis can help to identify systematic distortions of the instrument response when the underlying physical processes are well understood. Transient kinetics should always be determined at several different wavelengths, λ, and transient spectra at various delay times, t. Global analysis then provides the best fit of a trial reaction model to the matrix $\mathbf{Y}$ of

absorbance data $A(\lambda, t)$. These methods also find substantial application in fluorescence spectroscopy [8, 10].

Flash photolysis setups generally are single-beam instruments and, therefore, provide absorbance differences which may be positive or negative (bleaching). The precision achieved in measuring absorbance changes is surprisingly high ($\Delta A < 0.001$), as long as the measuring source remains constant over the measuring time interval. In other words, measurement of the pre-flash light level is essentially equivalent to "double-beam" operation by internal calibration. Serious problems arise only on long timescales (> 1 ms), where double-beam operation becomes a possibility.

The accurate measurement of fast transient waveforms requires correspondingly broad-band detection, which also picks up a broad spectrum of shot noise. To improve the signal/noise ratio in this situation one makes use of such expedients as intensified measuring light associated with low photomultiplier or amplifier gain, signal averaging, adjustable time constants (load resistors, filters), etc.

II. DATA GENERATION

II.1. Pump pulses

The duration of the pump pulse is one of the limiting factors that determine the time resolution of flash photolysis. The commonly used specification of pulse width at half height is incomplete, since the shape of many pulses is far from Gaussian. Tailing and afterglow of, e.g., conventional discharge flash lamps and excimer lasers, can severely reduce the time resolution. In this era of femtochemistry few groups still use the so-called "conventional" apparatus, i.e., flash photolysis systems pumped by a discharge flash lamp of microsecond duration. Yet, these instruments still give the best performance on the millisecond timescale and the whole setup costs a fraction of a laser.

II.2. Monitoring light sources and monochromators

High resolution in time requires high levels of the monitoring light in order to reduce shot noise, the random fluctuations in signal intensity that arise from the finite number of photons detected in short time intervals. Thus, xenon arcs are often pulsed to provide 10–100 times their continuous light output during a few milliseconds when used as monitoring light sources in nanosecond laser flash photolysis systems. Unfortunately, the poor reproducibility of the shape and intensity of the monitoring light pulses creates problems when weak signals require high amplification. Sophisticated electronics can provide highly reproducible, rectangular, current-limited pulses, but a substantial part of the light pulse variability remains due to arc instabilities and photomultiplier fatigue (*vide infra*). Thus, it is often preferable to use a brighter, stabilized xenon arc with good, spherically corrected optics and internal or external reflectors, and thereby avoid pulsing and the associated problems altogether. For target systems with small dimensions relative to the arc of the lamp such as the slits of a monochromator, the essential quantity is not the total power of the lamp, but the irradiance of its image at the target. The manufacturer's specifications should be consulted for the selection of light sources. For example, the brightness of a commercial 75-W xenon arc lamp (400 cd/mm^2) exceeds that of the 150-W arc (150 cd/mm^2) or of the 250-W arc (260 cd/mm^2) produced by the same manufacturer. When a lamp pulsing unit is installed, it should be turned off whenever slow events (τ > μs) are monitored; as the requirements for a fast rise time of the detector are relaxed, signal gain is boosted simply by increasing the terminal load on the photomultiplier.

Large slits will transmit more light and thereby improve the signal/noise ratio. But reduced spectral resolution is not the only drawback of large slit widths. Deviations from Beer's law result when the molar absorption coefficient of the absorbing species changes substantially over the wavelength interval transmitted by the monochromator and these deviations will lead to distortions of the kinetic traces [11]. This is usually not a serious problem with the diffuse absorption bands of most transient species in solution. Nevertheless, single wavelength kinetic traces are best measured at the absorption maxima of the transient species.

Photolysis induced by the monitoring light is unwanted but must be considered. In nanosecond laser setups the sample is shielded from the monitoring source by an electric shutter which opens shortly before

the laser pulse for a duration of a few milliseconds. The shutter not only reduces exposure of the sample but also of the photomultiplier and thereby reduces multiplier "fatigue". The sample can be further protected from the full intensity of the measuring beam by placing a narrow-band interference filter matched to the spectrometer setting in front of the sample. When measuring on timescales above 1 ms, the possibility that the measuring light may cause reversible absorbance changes needs to be considered. Transient absorbances with very long lifetimes should always be checked for a dependence on the measuring beam intensity.

II.3. Sample inhomogeneity, geometry of pump and probe beams

Most setups for transient absorption measurements are prone to produce transient concentrations which are unevenly distributed throughout the probed region of the sample. Inhomogeneities arise from hot spots in the laser beam profile, focusing optics, inner filter effects in the sample solutions, and imperfect overlap between the volume excited by the pump beam and that analyzed by the probe beam. Inhomogeneous sample distributions distort kinetics [12] and should be minimized, even at the expense of some loss in the signal intensity. It is obvious that inhomogeneous sample distributions will affect reaction kinetics of order two or higher. A gradient in the concentration at right angles to the monitoring beam will, however, distort even the traces of first- or zero-order reactions, since the average absorbance of such a solution will not be proportional to the average transient concentration.

Pump and probe beams are usually arranged either perpendicular or nearly parallel to each other. Strictly parallel orientation of the pump and probe beams is avoided in order to inhibit the strong pump pulse from hitting the detector of the probe beam. With laser excitation, a slight dealignment of the pump and probe beams is sufficient to eliminate the associated problems. Both orientations have some advantages and it is useful to devise the detection system such that it can be switched from one geometry to the other. Transient concentrations generated with the two setups may differ widely, and this will help to distinguish between first and second-order reactions.

Crossed beams (pumping perpendicular to probing) is the standard geometry used with conventional electric discharge flash lamps which are tube-shaped diffuse light sources. The sample cell is designed as a cylindrical or rectangular tube positioned in parallel between two or more excitation flash lamps of the same length (usually 10–20 cm). Right angle pumping is also advantageous when excimer lasers are used for pumping. Most excimer lasers give a rectangular output pulse covering an area of about 4 cm (horizontal) × 1 cm (vertical). Standard 1 × 1 × 4.5 cm^3 fluorescence quartz cells, modified to have an outlet tube on one of the sides and an optically flat window at the top, are ideally suited to capture the entire beam over a path length of about 4 cm (Fig. 1).

An advantage of right-angle excitation with diffuse pump beams is the relatively low concentration of transient intermediates produced in the sample. This reduces the usually unwanted bimolecular reactions between transient intermediates such as triplet-triplet annihilation. Sensitivity is maintained because the monitoring beam collects transient absorption over an extended optical path length. Three disadvantages of this setup are: (1) The monitoring light is strongly reduced at the wavelengths where the starting material absorbs, because the concentration of the substrate needs to be adjusted to obtain sufficient absorbance of the excitation pulse along the short excitation path length (direction y, Fig. 1) (2) A gradient in the concentration of the transient intermediates is generated along the excitation path (y), at right angles to the monitoring beam (direction -x), and this will result in distorted decay traces even for first-order reaction kinetics. (3) In order to minimize such distortions, one needs either to use solutions of low absorbance and waste a large fraction of the excitation pulse energy or to use narrow slits on the diaphragms in Fig. 1 and waste a large fraction of the monitoring light. A mirror placed at the rear end of the sample cell to reflect the transmitted fraction of the pump beam back through the solution, as shown in Fig. 1, has a twofold beneficial effect: it increases the fraction of absorbed light and it reduces the inhomogeneity of the transient distribution. Dielectric mirrors must be used because standard, surface-coated mirrors do not resist exposure to pulsed lasers. Scattering plates covered with $BaSO_4$ or MgO may serve the same purpose, but are a hazard to the unprotected eye.

An advantage of parallel pump and probe beams is that the sample absorbance can be raised to a point where essentially all of the exciting light is absorbed. On-axis pumping is to be preferred in a study

requiring variation of the concentration of an additive such as a quencher which absorbs part of the excitation pulse. In such a case the concentration of the photo-substrate and the additive can be varied in proportion over a wide range without affecting the fraction of light absorbed by the substrate. On-axis pumping with total absorption of the excitation pulse will, however, produce high transient concentrations and thereby favor bimolecular reactions between transient species.

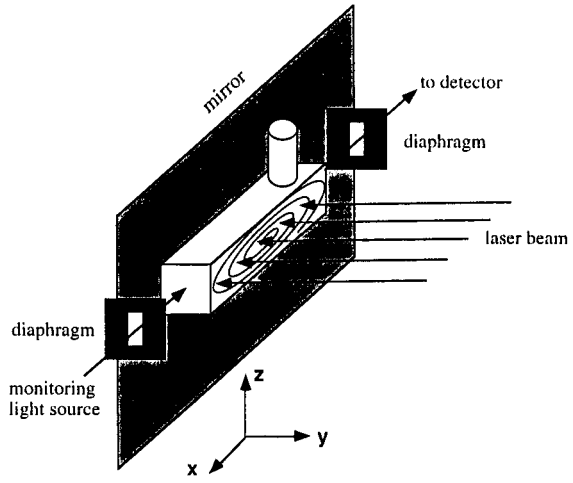

Figure 1. Perpendicular pumping with rectangular excimer laser beams

When the number of photons absorbed in the solution is of the same order of magnitude as the number of absorbing molecules, it is necessary to use a time-dependent, differential form of the Beer-Lambert law in order to calculate transient distributions along the path of the excitation pulse. For example, in a 10^{-5} M solution of a strongly absorbing dye, $\varepsilon = 10^5$ M^{-1} cm^{-1}, a weak light beam is attenuated to 10% of its initial intensity over a path length of 1 cm. However, the 100-mJ pulse of an excimer laser may well penetrate the same solution without much attenuation, because 15% of its photons are sufficient to excite all of the dye molecules. Conversely, a laser pulse may be more strongly absorbed than a weak light beam of the same wavelength, if the initially absorbing species generates more strongly absorbing transient intermediates on excitation.

The distortions on the observed kinetic traces which must be expected from the generation of inhomogeneous sample distributions can be calculated for a given model reaction by numerical integration of the coupled differential equations describing (*i*) the fraction of the pump pulse absorbed, (*ii*) the resulting concentration changes, and (*iii*) the contribution to the transient absorption at λ_{obs} in a thin slice of the probe. As a rule of thumb, distortion of the kinetics from inhomogeneous sample distributions is insignificant with right-angle excitation as long as both the initial absorbance at the excitation wavelength and the transient absorbance at the monitoring wavelength are kept below 0.2. With strongly absorbing chromophores, higher absorbances are tolerable at the excitation wavelength when the number of photons in the pump pulse exceeds the number of molecules in the probe volume.

The effect of inhomogeneous sample distributions for an individual setup can be determined empirically by observing a strongly absorbing transient intermediate with a clean first-order decay such as the triplet of 9,10-dibromoanthracene in aerated solution. A sigmoid trend (saturation of the initial absorbance with respect to the expected exponential decay) eventually becomes conspicuous as the absorbances of the starting material and of the transient are increased.

II.4. Photoselection with polarized pump pulses

The output of most lasers is polarized to some extent. Excited molecules and primary photoproducts will therefore be formed preferentially in orientations for which the transition moment of the starting material is parallel to the polarization of the pump pulse (photoselection). If the monitoring light is also polarized, its direction of polarization should be set at the "magic" angle of 54.74° relative to that of the pump pulse in

order to eliminate absorbance changes arising from rotational depolarization after pumping. Depolarization occurs on the timescale of rotational diffusion, that is within a few picoseconds with small molecules in solvents of low viscosity, but can be much longer in highly viscous media or organized systems (micelles, membranes, etc.).

II.5. Temperature control

Good kinetic data are usually reproducible to within 5%. A systematic change of 5% will result from a change in temperature of 1 °C for a reaction with an activation energy of, e.g., E_a = 40 kJ mol^{-1}, a common value for rates on the order of 10^6 s^{-1}. Thus, sample cells must be held in a thermostat if quantitative and reproducible kinetic data are required.

II.6. Shock waves

At high flash intensities or excessively high absorbance of the sample, thermally induced shock fronts may be formed and reflected around in the sample cell. These are easily recognized by their complex transmission profiles showing erratic oscillations with periods of about 10 μs.

III. DATA COLLECTION AND STORAGE

III.1. Kinetic detection systems

Standard side-on photomultipliers with rise times of ca. 2 ns are mostly used to monitor transmission changes in time at a fixed wavelength. Since high light fluxes are required to avoid shot noise, amplification of the photoelectrons in the photomultiplier tube is usually limited to five dynodes [13]. The capacitance of the photomultiplier wiring must be kept as low as possible. With a terminal load R of 50 Ω, the capacitance C must be below 40 pF to keep the RC time constant of the detection system below 2 ns. Signal amplitudes should be held below about 200 mV to avoid saturation effects (photomultiplier fatigue). The signal current reaches 4 mA at an amplitude of 200 mV on 50 Ω. Even higher currents may be sustained with no loss in performance on the microsecond timescale. However, photomultiplier responses do show marked fatigue (drift) on millisecond timescales at such high light levels [13]. This is why exposure of the photomultiplier to the monitoring source should be minimized with the aid of an electronic shutter. When a reproducible pretrigger level is required at high signal amplification, the photomultiplier output can be measured, digitized, and the voltage offset balanced shortly before the laser pulse. However, such devices add to the capacitance and, hence, to the risetime of the detection system.

Reflections in the cable connecting the photomultiplier to the amplifier may lead to signal distortions on short timescales, notably when very abrupt changes occur initially due, e.g., to stray light (cf. section III.3). Such reflections can be reduced by terminating both ends of the cable with the same load. Of course, a 50 Ω load on both ends of the cable is equivalent to 25 Ω total resistance and is associated with a corresponding loss in sensitivity.

Digitizing oscilloscopes with sampling rates of ≥ 1 Gigasample/s *and* high-bandwidth amplifiers (≥ 500 MHz) nicely match the time resolution required to see the fastest processes resolvable by excitation with Q-switched lasers which have pulse half-widths on the order of 10 ns. The time base (horizontal) and amplifier (vertical) settings must be adapted to the expected signal to ensure maximum resolution. The high storage depth of modern digitizers provides easy access to long delay times even at high time resolution such that both fast and slow processes may be captured in a single trace. Furthermore, the pre-trigger capturing facility of these devices obviates the need for early trigger signals. Jitter is virtually eliminated when the trigger is generated directly from the laser pulse with a photodiode.

III.2. Spectrographic detection systems

The photographic plates used to record the absorption spectra of transient intermediates have been replaced by diode arrays and vidicon detectors. The time window, during which the transient spectra are collected, can be determined by employing a short probe pulse of light. Alternatively, microchannel plate image

intensifiers placed in front of the diode arrays may be used in combination with continuous monitoring light sources to achieve nanosecond time resolution; image intensifiers not only enhance the intensity of the monitoring light during the active time window, but also act as shutters with a (de-)activation rise time of ca. 1 ns and an on:off transmission ratio > 10^6. Such an effective shutter is required if the monitoring light source is continuous during the long integration times of the detectors (ca. 30 ms). Cut-off filters should be used for protection of the detector from the excitation pulse and to eliminate second-order reflections from the grating. When excess monitoring light is available at some wavelengths, compensating filter solutions may be adjusted with appropriate dyes to equalize the spectral distribution of the source.

III.3. Light scattering

Fluorescence will interfere with kinetic absorbance measurements for the duration of the fluorescence or of the pump pulse, whichever is longer. Scattered light and fluorescence can easily drown the monitoring light source because the intensity of the pump pulse is commonly orders of magnitude higher than that of the probe pulse or of the continuous monitoring light source, respectively. For example, a dye with a fluorescence yield of 10%, which is excited by a laser pulse of 5 ns duration and an energy of 5 mJ, will produce close to 0.5 mJ of fluorescence distributed in all directions over, say, a spectral width of 25 nm and a time window of 10 ns. If the fluorescence is collected over a solid angle of 5 ° (the aperture of a "good" monitoring beam), the spectral flux amounts to about 1 W/nm. For comparison, the spectral flux of the monitoring beam from a 75-W xenon arc with good optics is estimated to be on the order of 1 mW/nm from spectral irradiance specifications provided by lamp suppliers.

"Stray light" problems are reduced by using appropriate filters, by pulsing the monitoring lamp, and by taking advantage of the widely different angular dependence's of scattered light and the monitoring beam. The dispersion of the monitoring light beam may be minimized with improved optics or by using a laser probe beam. The collecting lens can be positioned at some distance behind the sample cell. Further discrimination is possible by limiting the diameter of the collecting lens with an iris and by reducing the slit heights at the monochromator entrance and exit to those required to transmit the probe beam only. When the stray light is due to impurities, photoproducts, suspended particles in solution, or even to the cell material and the sample holder, it may be reduced by appropriate measures. In case of severe scattering, the photomultiplier may be protected from overload by gating of the cathode voltage.

III.4. Signal averaging

Noise levels are intrinsically high in flash photolysis for several reasons: (a) Electronic filtering of high-frequency noise is limited by the demand for high-frequency response in capturing fast events. (b) Shot-noise (poor photon statistics) is associated with the demand for high time resolution. (c) Flash photolysis setups are, with few exceptions, single-beam and, therefore, do not compensate for fluctuations of the monitoring light beam. In kinetic setups this results in base line instabilities, especially on long timescales. The determination of transient spectra in spectrographic setups requires a reference light pulse which is either generated at a different time or is passed through a different (non-pumped) region of the sample cell.

Data averaging reduces random noise and can thereby provide increased signal/noise ratios. As increasingly short laser pulses are used to improve the time resolution, the capability to detect weak signals from low-energy pulses gains in importance, since high-power pump pulses are often associated with (sequential or simultaneous) two-photon absorption. High-energy pulses also give rise to diffusive encounters between highly reactive intermediates which may induce chemical events not encountered with continuous light sources. Such events are considered as unwanted "artifacts" when the research goal is to elucidate the reaction mechanism under "normal" irradiation conditions.

Most modern digitizers offer the possibility to accumulate (and thereby average) the output signal directly in the memory. Direct averaging is appropriate only when the jitter of the triggering signal with respect to the excitation pulse is much shorter than the lifetime of the transient intermediates, and when both the energy of the excitation pulse and of the analytical light level are highly reproducible. The requirement for reproducible excitation and monitoring light pulses arises because the signal intensity is proportional to optical transmittance T and must be converted to absorbance, $A = \log(1/T)$, for subsequent

analysis. Errors arising from performing this transformation on the accumulated signal, rather than on each individual trace, will be small when the transient absorptions are very small in amplitude. However, it is best to avoid such problems by transferring each trace to the computer for accumulation after transformation. This is generally no problem at repetition frequencies of 10 Hz or less, and even allows the incorporation of checking routines to eliminate unsatisfactory traces from accumulation.

III.5. Filtering

A simple way to reduce the high-frequency noise is to pass the signal through an electronic device with a limited response bandwidth. As a rule of thumb, the rise time of the electronically filtered signal should be at least 10 times faster than the rate constant of the fastest process to be monitored. The *RC* filter, which attenuates noise components with frequencies higher than $1/(2\pi RC)$, is simple, cheap, and easy to adjust as a function of the required time resolution. This type of filter is commonly used but it must be pointed out that its frequency cut-off properties are not very sharp: for instance, if the attenuation factor is 1.5 for a given frequency ν, it will be 2 for 2ν and 3 for 4ν. More efficient electronic filters with much sharper characteristics are mass produced for the telecommunications industry, but they need to be carefully tested for signal distortions.

Electronic filters were essential to improve signal/noise ratios as long as the experimental data were recorded on analog oscilloscopes, since signal averaging was not available. The importance of electronic filters has decreased due to the increasing use of transient digitizers and digital oscilloscopes. It is recommended to use the largest available storage depth coupled with a fast sampling rate. Whether the full set of data is processed by least squares minimization or after an appropriate method of data reduction (see below) is not important; in both cases the signal/noise ratio will increase with the square root of the number of data points sampled originally.

Electronic filtering down to about the sampling frequency of transient oscilloscopes is advantageous, because these devices create a record by saving an "instant" sampling value, not one obtained by averaging over the entire acquisition interval. Some amplifiers offer reduced bandwidth options for this purpose. A high resolution option, which stores an average of several samples taken during the acquisition interval, is available on some digitizers, but is effective only for slow sweep rates. Notice that analog oscilloscopes automatically filter noise having frequencies much higher than the reciprocal of the sweep rate: the writing intensity can be diminished such that high-frequency noise giving rise to fast vertical movements is not recorded. This is also true for devices such as the Tektronix 7912 which digitize data from an analog representation of the signal.

III.6. Data reduction

A digitized trace usually consists of at least 500 data points; storage depths of 50,000 points and more are currently becoming standard. An essential first step for data analysis is the (arbitrary) choice of the "useful" part of the decay curve. It is advantageous to choose a time window of at least five half-lives for analysis, such that the end absorbance is accurately defined. Restriction to shorter times may be required when the baseline shows large fluctuations, as is often the case on relatively long timescales, especially with pulsed light sources. Reproducible baseline drifts should be eliminated by subtraction of a reference blank trace.

Efficient software for nonlinear least-squares fitting of simple rate laws with one or two nonlinear parameters (the rate constants) to 5000 absorbance data points requires less than a minute on a personal computer. Therefore, it is seldom necessary to reduce the number of data points prior to analysis. If data reduction is required to avoid excessive computing time, it is sufficient to reduce the grid density by progressive linear interpolation in small intervals as long as the signal is represented by at least 100 data points. The use of a variable grid density for the reduced data set in order to capture more detail of the signal where it varies most is not recommended, since the use of variable interval lengths for data averaging introduces changes in the variance of the reduced data points. Proper consideration of the variance changes by weighting would then produce essentially the same result as using an equidistant grid.

III.7. Arrays of 2-dimensional data

Most flash photolysis systems designed for nanosecond and longer timescales operate in the kinetic mode, i.e., the transient absorption is monitored at a single wavelength as a function of time. Those designed for picosecond and femtosecond time resolution usually operate in the spectrographic mode and provide digitized transient absorption spectra at a given time delay with respect to the laser pulse. Basically, both techniques probe uni-dimensional slices of the same physical information that consists of a two-dimensional array **Y** of absorbances, $A(\lambda, t)$, as a function of wavelength, λ, and time-after-excitation, t.

Streak cameras allow one to probe the entire two-dimensional array $A(\lambda, t)$ after excitation with a single pulse. They are, however, rarely used for pump-probe flash photolysis because of their low precision and high price. Repeated excitation is necessary to probe different wavelengths on a kinetic apparatus, and different time delays on spectrographic devices. Variations of the pump pulse intensity will affect the spectral information determined point-by-point in the kinetic mode, and the kinetic information derived from sequential spectra. Systematic variations of the instrument performance or decomposition of the sample with time can easily produce artificial kinetics in the spectrographic mode and artificial spectral features in the kinetic mode, respectively. It is, of course, best to determine and eliminate the source of such problems. Notwithstanding, probing at different wavelengths or time delays should always be done at random rather than in an ordered sequence; if systematic variations remain, they will produce an increase in apparently random noise rather than artificial features.

If possible, fluctuations should be eliminated by calibration, i.e., by independent monitoring of the pump pulse intensity for each individual shot. Sample degradation can be avoided by using flow systems. In picosecond systems, where the time delay between the arrival of the pump and probe pulses at the sample is set by an optical delay line, it is essential to assure that the overlap of the two pulses on the sample does not change with the time setting. Proper alignment should be tested routinely by monitoring some absorption which is generated by the pump pulse and which is known to persist throughout the time range of the delay line. Any seeming growth or decay of that absorption would then indicate misalignment of the two pulsed beams.

IV. DATA ANALYSIS

No mathematical treatment can ever make up for less than optimal methods of data collection. Similarly, it is preferable to simplify the reaction kinetics by choosing appropriate conditions rather than to analyze a complex system with sophisticated mathematical methods. For example, when one has a mixture of first- and second-order kinetics, attenuating the light dose to the point where only the first-order kinetics are important yields more reliable results (in spite of the decreased signal/noise ratio) than a mathematical separation of the two processes.

IV.1. Analytical versus numerical integration of differential rate equations

Rate laws derived from reaction schemes consisting of any number and combination of consecutive and parallel first-order reaction steps can be integrated in closed form to give a sum of exponential terms, eqn. (1) [14].

$$A_{obs}(t) = A_0 + A_1 \exp(-k_1 t) + A_2 \exp(-k_2 t) + \ldots \quad (1)$$

Models involving parallel first- and second-order reactions also have straightforward analytical solutions [15]. Numerical integration methods are rarely required, since, for the fast reactions of interest here, the majority of cases encountered will be covered by the above choice. The computer-assisted analysis of kinetic data is intrinsically numerical, irrespective of whether concentrations are calculated using an analytical function or determined by an iterative numerical procedure from the rate constants. Calculation times needed for iterative fitting to differential rate equations (Runge-Kutta, Bulirsch-Stoer, Gear, Enright, Rosenbrock, Adams [16]) are, however, one or two orders of magnitude larger than those for nonlinear least-squares fitting to analytical functions. Numerical integration will thus be the method of choice only as an exception rather than as a rule. Nevertheless, its accuracy is in general satisfactory and general

numerical integration routines even offer some advantages: The program input used to define the kinetic model (the set of differential equations) can be used directly and there is no need to implement the program with varying computer code for specific integrated forms. A versatile program for the analysis of kinetic data should thus include a numerical integration routine as an option, besides the most common integrated functions for a series of first-order relaxations and for a simple second-order reaction. While the various numerical integration routines all have their specific advantages [16], the predictor-corrector method first described by Gear [17] is frequently used in chemical kinetics because it is capable of handling very stiff differential equations, i.e., models with rate constants differing by several orders of magnitude.

Differential analysis of kinetic data, i.e., the evaluation of rate constants by fitting the rate equations derived from the reaction model directly to experimental slopes, requires data smoothing and is not recommended in general, because the functions used for smoothing tend to introduce systematic error. This is true in particular for polynomials [18]; exponential terms would seem intrinsically better suited to simulate kinetic traces [19]. While it is true that such methods are faster than numerical integration, that advantage is losing importance with the increasing speed of computing becoming available cheaply.

IV.2. Nonlinear least squares fitting and elimination of linear parameters

The response of photomultipliers, photodiodes and diode arrays is linear in light flux within a specified range. Within this range, the readings will be proportional to the transmittance T of the sample. Noise associated with the dark current is commonly negligible, since integration times are short. Under these circumstances, the *relative* error of the transmittance, and the *absolute* error of the sample absorbance, calculated as $A = \log(1/T)$, will be constant. Minimization of the squared residuals, χ^2, with respect to a model function such as eqn. (1) should therefore be done with absorbance data where each data point properly receives equal weighting. It is not recommended to transform the data points in order to linearize the model function, e.g., to $\log(A)$ for first-order reactions or to A^{-1} for second-order reactions, because the associated errors transform accordingly. Transformation of the data would then require the use of appropriate weighting factors which can only be determined iteratively [20], forsaking any advantage gained by linearization.

Therefore, the trial model function will in general be a nonlinear function of the independent variable, time. Various mathematical procedures are available for iterative χ^2-minimization of nonlinear functions. The widely used Marquardt procedure is robust and efficient [21]. It is less commonly realized that not all the parameters in the model function need to be determined by iteration. Any kinetic model function such as eqn. (1) consists of a mixture of linear parameters, the amplitudes of absorbance changes, A_i, and nonlinear parameters, the rate constants, k_i. For a given set of k_i, the linear parameters A_i can be determined without iteration (as in any linear regression) and they can, therefore, be eliminated from the parameter space in the nonlinear least-squares search [22]. This increases the reliability in the determination of the global minimum and reduces the required computing time considerably. It is worth applying the algorithm of linear parameter elimination even in the most simple schemes, e.g., for a single exponential with constant terms, where the number of iteratively refined parameters reduces from 3 to 1.

IV.3. Mechanistic ambiguity of mathematical model functions

While analytical model functions (closed integral forms of the differential kinetic equations) *are* available for most reaction schemes encountered in fast kinetics, it is by no means trivial in general to derive the physically relevant elementary rate constants and the related concentration profiles from the model parameters determined by the fit. Consider the systems that are mathematically described by a sum of exponentials (eqn. 1). Such a mathematical model identically fits to mechanisms consisting of a series of parallel first-order reactions or to any combination containing parallel, consecutive and reversible monomolecular elementary steps. In such a situation of mathematical ambiguity it is essential to consider and make use of any additional information or insight that may be available. The external information may consist of mechanistic considerations based on chemical intuition or prior independent knowledge of individual molar absorption coefficients and/or rate constants. Last, but not least, distinctions between acceptable and unacceptable mechanisms may be possible on the basis that the absorbances of all species must be non-negative throughout the spectrum [23], and that the concentrations of all species must be non-negative at all times.

First-order rate constants of transients which are affected by second-order contributions of dilute species cannot be determined reliably, even when attempts to correct for the observable second-order contributions are made. A typical example is the triplet state lifetime of anthracene in degassed solution which appears to be somewhere in the range of 20 μs to 1 ms on most instruments (values are reproducible on a given instrument but vary strongly between different setups and solvent purification methods). The intrinsic first-order lifetime is at least 25 ms when quenching by diffusional encounters with the parent molecule (self-quenching), with other molecules in the triplet state (triplet-triplet annihilation), and with impurities is carefully reduced (chemical deoxygenation) [24].

A special situation arises for consecutive reactions with very similar rate constants. It is rather obvious that two parallel reactions, $A \rightarrow B$ and $C \rightarrow D$, in the limit $k_{AB} = k_{CD}$ will lead to a single exponential obscuring the existence and the relative amplitudes of the two individual steps. Less obviously, the same problem pertains to consecutive reactions, $A \rightarrow B \rightarrow C$. If only B absorbs at the wavelength of observation then a rise in absorbance will be followed by a decay and it is immediately apparent that a consecutive reaction is being observed. Nevertheless, because of the mathematical identity of the rate law with that of the previous example, even such a curve will be physically ambiguous in the case of closely similar rate constants. Nonlinear least-squares fitting will be difficult or impossible because of near-singularity of the Hessian matrix (the second derivative of the χ^2 merit function [21b]) and extreme correlation between rate constants and molar absorption coefficients. Reliable analysis is possible only if additional information is available regarding either the spectral characteristics of the species involved or one of the rate constants.

IV.4. Weighting. Determination of bimolecular rate constants from the concentration-dependence of first-order rate coefficients (Stern–Volmer Analysis)

A common procedure in mechanistic studies is to follow the growth or decay rate of a transient species as a function of the concentration of some other species such as a sensitizer, quencher, trapping agent, acid, buffer, etc. Bimolecular rate constants are then obtained by least-squares fitting of the appropriate rate law to the observed first-order rate constants, k_{obs}, as a function of the concentration of the interacting species. Usually, the concentration of the interacting species is known accurately and is treated as the independent variable.

It is, in general, not good practice to use transformed rate laws, such as the frequently encountered "dual reciprocal plot", which provide a linear dependence on the independent variable. Proper analysis of such data should take account of the following two considerations: a) The accuracy in the determination of a given first-order rate constant will depend on the value chosen for the independent variable. If the data points are easily available, it is best to determine the variance in k_{obs} for each value of the independent variable. The resulting estimated sample variances may then be used to weight each averaged data point by the inverse of its estimated variance. In many cases, a reasonable approximation is to assume that the *relative* error of k_{obs} is constant. Instead of introducing appropriate weights by estimating absolute errors from the absolute values of k_{obs}, least-squares fitting should then be done to the logarithm of k_{obs}, since the error in $\log(k_{obs})$ will then be constant. However, other dependence's may well arise. The signal amplitude may be a function of the reactant concentration when the observed signal arises from trapping of an invisible transient intermediate, as is the case when a carbene is monitored by the formation of an ylid [25]. If weighting based on sufficiently large data sets at each concentration is not feasible, then it should be replaced by weighting based on an appropriate *a priori* estimate of the sample variances.

b) Standard least squares analysis may yield a negative rate constant or a confidence interval extending to negative values, when the intercept of a steep linear regression is poorly defined. If one wants to implement the knowledge that rate constants cannot have negative values in the statistical analysis, one is dealing with the situation of a regression with constraints in the form of inequalities, $k \geq 0$. The Langrange method of undetermined multipliers is applicable only to situations where the constraints can be expressed in the form of equations. Gelfand *et al.* [26] have shown that the posterior distribution of the parameters in a Bayesian approach can be derived using a Gibbs sampling method. This allows one to use the unconstrained conditional distributions restricted to the subset of allowed parameter values and thus to obtain the (skewed) posterior distributions, their modes and 95% density regions, which are restricted to

the physically meaningful range of values, for each of the model parameters. An application program for Gibbs sampling of model functions is available as freeware [27].

IV.5. Actinometry

It is always difficult but often essential to determine absolute transient concentrations, and, hence, molar absorption coefficients and quantum yields of formation. The methods have been critically reviewed and some reference standards were recommended [28]. The determination of triplet molar absorption coefficients by the method of total singlet-triplet conversion is still best achieved using old-fashioned "conventional" flash photolysis which permits many excitation cycles within the flash lifetime, with resulting accumulation of excited molecules in the triplet level [29]. A number of studies have been devoted to calibrate chemical actinometers for use in flash photolysis [30].

IV.6. Visualization

The graphical display of data is an essential part of data analysis, pattern recognition and for the judgment of fits from the trends in residuals, etc., but will not be treated here.

V. ARRAYS OF DATA: GLOBAL ANALYSIS AND FACTOR ANALYSIS [31]

V.1. Global analysis [32]

Global, i.e., simultaneous analysis of a complete $W \times M$ array $\boldsymbol{Y}$ of absorbances $A(\lambda, t)$ (W: number of wavelengths λ studied, M: number of measurements at delay time t per kinetic trace) should be considered for all but the simplest reaction models. Multiwavelength data will generally lead to a more accurate determination of the relevant rate constants. The determination of the absorption spectra of the individual species participating in a reaction by global analysis helps to distinguish between different mechanistic models. The elimination of linear parameters becomes a must for multiwavelength data, as it will be impossible to treat all unknown molar absorption coefficients at the different wavelengths as independent and unknown variables. Almost invariably, convergence would fail for molar absorption coefficients at certain wavelengths and on the other hand it would be extremely tedious if not impossible to obtain the overall 'best' rate constants through stepwise analysis of single wavelength data. With global analysis, the number of iteratively refined parameters remains equal to the number of rate constants, and is independent of the number of wavelengths used. In fact, the recommended [22] least-squares algorithm is practically identical for single- and multiwavelength data. For either case the residuals between the model function and the experimental data are given by eqn. (2), in matrix notation

$$\boldsymbol{R} = (\boldsymbol{C}^t\boldsymbol{C})^{-1}\boldsymbol{C}^t\boldsymbol{Y} - \boldsymbol{Y}. \tag{2}$$

For single wavelength data, the $M \times W$ matrix of absorbances simply reduces to a vector of dimension M. The concentration matrix $\boldsymbol{C}$ is uniquely defined by the set of rate constants and is independent of the number of wavelengths monitored.

V.2. Factor analysis [31]

Factor analysis (FA) provides an efficient tool for data reduction, increasing computational efficiency, and model selection. In addition, the use of FA may lead to significant elimination of random noise. The goal of FA, which is also called principal component analysis or target factor analysis, is to determine directly, and on a completely model-independent basis, the number of absorbing species in the reaction system. FA is not a panacea in this respect, however: linearly dependent spectra or essentially parallel concentration profiles will lead to the loss of a significant factor, whereas instrumental instabilities will produce artificial ones.

For FA, the original data set $\boldsymbol{Y}$ is subjected to singular value decomposition (SVD), by decomposition of $\boldsymbol{Y}$ into a product of three matrices, $\boldsymbol{U}$, $\boldsymbol{S}$ and $\boldsymbol{V}$, eqn. (3)

$$Y = U S V = L V \quad (3)$$

S is a diagonal matrix with the "singular values" (the square roots of the eigenvalues of the second moment matrices Y^tY or YY^t) as entries. Only the significant singular values are retained, the other diagonal elements of S are set to zero. Least-squares refinement is then done on the loading matrix $L = U \times S$ instead of Y, which theoretically, i.e., in the absence of noise or in the limit of perfectly random noise, leads to the identical minimum as would have been obtained with the full data set. Concomitantly, the size of the relevant matrix is greatly reduced, typically from 50 × 500 for Y to 50 × 3 for L (assuming 3 colored species). Although the complete iterative refinement is done in the abstract eigenvector space, no kinetic or spectral information is lost and after refinement the real spectra may be simply and non-iteratively obtained by subsequent rotation of the abstract spectra upon matrix multiplication with the eigenvectors V.

Finally, SVD provides access to all the chemometric methods of model-free determination of concentration profiles and absorption spectra by variants of target factor analysis, e.g., ensuring non-negativity of concentrations and absorbances or by evolving factor analysis [33]. Such model-free tools are not intended to replace the fitting of appropriate model functions. Rather, they are useful as tests whether a given model is "forced to" or naturally emerges from a given set of data. It is gratifying if the model-free analysis is consistent with that based on a chemically reasonable model.

V.3. Second-order globalization [34]

With the increase in speed and memory resources of personal computers, second-order globalization has become a feasible approach for on-site analyses. Here, not only a complete $M \times W$ data array is refined at once, but several such sets are combined to a single superset. Parameters to be refined then no longer are individual rate constants. Rather, experiments done at, e.g., different temperatures or with different reagent concentration are processed and activation parameters or second-order rate constants underlying a set of first-order coefficients are obtained as the optimum values in a single step. It should be kept in mind, however, that such analyses are based on assumptions such as the independence of absorption spectra on temperature or reagent concentration and that such assumptions can lead to erroneous conclusions. An instructive example is given in a recent paper by Saltiel and coworkers [35]; the authors show that conformational analysis, based on absorption spectra obtained at different temperatures and the (false) assumption that the absorption spectra of the individual conformers are independent of temperature, leads to erroneous conclusions. Used diligently, the global analysis of multidimensional digital data is a powerful tool in modeling which may become standard in kinetics and elsewhere.

REFERENCES

[1] R. G. Norrish and G. Porter, *Nature* **164**, 658 (1949).

[2] G. Porter and M. A. West in: "Techniques of Chemistry", Vol. 6, Part 2 (G. G. Hammes, ed.), Wiley-Interscience, 367 (1974).

[3] "CRC Handbook of Organic Photochemistry", J. C. Scaiano, ed., Vol. I, CRC Press, Boca Raton, 1989; (a) L. M. Hadel (chapter 11: Laser Flash Photolysis); (b) F. Wilkinson and G. P. Kelly (chapter 12: Diffuse Reflectance Flash Photolysis); (c) S. M. Hubig and M. A. J. Rodgers (chapter 13: Picosecond Absorption Spectroscopy).

[4] "Lasers in Polymer Science and Technology: Applications", J.-P. Fouassier and J. F. Rabek, eds., CRC Press, Boca Raton, 1990; (a) D. Phillips and G. Rumbles (Vol. I, chapter 3: Time-Resolved Laser Spectroscopy Nanosecond and Picosecond Techniques, Principles, Devices, and Applications); (b) F. Wilkinson and C. J. Willsher (Vol. II, chapter 9: Diffuse Reflectance Laser Flash Photolysis of Dyed Fabrics and Polymers).

[5] (a) R. V. Bensasson, E. J. Land, and T. G. Truscott, "Flash Photolysis and Pulse Radiolysis", Pergamon Press, New York, 1983. (b) "Primary Photo-Processes in Biology and Medicine", R. V. Bensasson, G. Jori, E. J. Land, and T. G. Truscott, eds., Plenum Publ. Co., New York, 1985. (c) R. V. Bensasson, E. J. Land, and T. G. Truscott, "Excited States and Free Radicals in Biology and Medicine, Contributions from Flash Photolysis and Pulse Radiolysis", Oxford University Press, 1993. (d) "Ultrafast Processes in Chemistry and Photobiology", M. A. El-Sayed, I. Tanaka, and Y. Molin, eds., Blackwell Science, Oxford, 1995.

[6] J. C. Scaiano, M. Tanner, and D. Weir, *J. Am. Chem. Soc.* **107**, 4396 (1985). A. Longoni and G. Ponterini, *Appl. Spectroscopy* **40**, 599 (1986). N. P. Ernsting and M. Kaschke, *Rev. Sci. Instrum.* **62**, 600 (1991). C. J.

Beischel, S. K. Moore, R. K. Crouch, and D. R. Knapp, *Rev. Sci. Instrum.* **63**, 2069 (1992). E. Hasler, A. Hörmann, G. Persy, H. Platsch, and J. Wirz, *J. Am. Chem. Soc.* **115**, 5400 (1993).

[7] F. Wilkinson, *J. Chem. Soc., Faraday Trans. 2*, **82**, 2073 (1986). D. Oelkrug, W. Honnen, F. Wilkinson, and C. J. Willsher, *J. Chem. Soc., Faraday Trans. 2*, **83**, 2081 (1987). F. Wilkinson and R. Beer in: "Photochemical Processes in Organized Molecular Systems", K. Honda, ed., 377 (1991).

[8] D. F. Eaton, *Pure Appl. Chem.* **62**, 1631 (1990).

[9] T. T. Ndou and I. M. Warner, *Chem. Rev.* **1991**, 493 (1991).

[10] J. van Stam, L. Van Dommelen, N. Boens, K. Zachariasse, and F. C. De Schryver, *J. Phys. Chem.* **99**, 9386 (1995).

[11] Page 425 of ref. [2]; formula (10.13) should read $OD_{app} = \log\{(1+x)/[\exp(-\varepsilon_1 cl) + x \exp(-\varepsilon_2 cl)]\}$.

[12] J. W. Boag, *Trans. Faraday Soc.*, **64**, 677 (1968). M. Bazin and T. W. Ebbesen, *Photochem. Photobiol.*, **37**, 675 (1983)

[13] A. Fenster, J. C. LeBlanc, W. B. Taylor, and H. E. Johns, *Rev. Sci. Instrum.*, **44**, 689 (1973).

[14] Cf., e.g., S. W. Benson, "The Foundations of Chemical Kinetics", McGraw-Hill: New York, 1960.

[15] C. Capellos and B. H. J. Bielski, "Kinetic Systems", Wiley: New York, 1972.

[16] G. Engeln-Müllges and F. Reutter, "Numerische Mathematik für Ingenieure", BI, Mannheim, 1985.

[17] C. W. Gear, *Comm.* ACM, **14**, 176, 185 (1971).

[18] C. Steel and K. R. Naqvi, *J. Phys. Chem.*, **95**, 10713 (1991).

[19] J. P. Guthrie, *Can. J. Chem.*, **60**, 765 (1982). L. J. Andrews, J. M. Levy, and H. Linschitz, *J. Photochem.*, **6**, 355 (1976/77).

[20] D. E. Sands, *J. Chem. Ed.* , **7**, 473 (1974). S. D. Christian, E. H. Lane, and F. Garland, *J. Chem. Ed.*, **7**, 475 (1974).

[21] (a) D. W. Marquardt, *J. Soc. Ind. Appl. Math.*, **11**, 431 (1963). (b) W. H. Press, B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling, "Numerical Recipes", Cambridge University Press, Cambridge, 1990.

[22] G. H. Golub and V. Pereyra, *SIAM J. Numer. Anal.*, **10**, 413 (1973). H. Gampp, M. Maeder, and A. D. Zuberbühler, *Talanta*, **27**, 1037 (1980).

[23] W. H. Lawton and E. A. Sylvestre, *Technometrics*, **13**, 617 (1971).

[24] H. Linschitz, C. Steel, and J. A. Bell, *J. Phys. Chem.*, **66**, 2574 (1962). K. H. Grellmann and H.-G. Scholz, *Chem. Phys. Letters*, **62**, 64 (1979).

[25] B. R. Arnold, J. C. Scaiano, G. F. Bucher, and W. W. Sander, *J. Org. Chem.*, **57**, 6469 (192). J.-L. Wang, J. P. Toscano, M. S. Platz, V. Nikolaev, and V. Popik, *J. Am. Chem. Soc.*, **117**, 5477 (1995).

[26] A. E. Gelfand, A. F. M. Smith, and T.-M. Lee, *J. Am. Stat. Ass.*, **87**, 523 (1992). G. Casella and E. I. George, *The American Statistician*, **46**, 167 (1992).

[27] D. Spiegelhalter, A. Thomas, N. Best, and W. Gilks, BUGS (Bayesian inference Using Gibbs Sampling), MCR Biostatistics Unit, Institute of Public Health, Robinson Way, Cambridge CB2 2SR, UK, bugs@mrc-bsu.cam.ac.uk.

[28] R. Bonneau, I. Carmichael, and G. L. Hug, *Pure & Appl. Chem.*, **63**, 289 (1991). See also: A. Yoshimura, M. Z. Hoffman, and H. Sun, *J. Photochem. Photobiol. A*, **70**, 29 (1993). L. Biczok, H. Linschitz, and R. I. Walter, *Chem. Phys. Lett.*, **195**, 339 (1992).

[29] L. Pekkarinen and H. Linschitz, *J. Am. Chem. Soc.*, **82**, 2407 (1960). I. Carmichael and G. L. Hug, *J. Phys. Chem.*, **89**, 4036 (1985).

[30] S. Takamuku, G. Beck, and W. Schnabel, *J. Photochem.*, **11**, 49 (1979). G. Gauglitz and S. Hubig, *J. Photochem.*, **15**, 255 (1981). K. Murata, Y. Yamaguchi, H. Shizuka, and S. Takamuku, *J. Photochem. Photobiol. A*, **60**, 207 (1991). J. C. Mialocq, X. Armand, and S. Marguet, *J. Photochem. Photobiol. A.*, **69**, 351 (1992).

[31] E. R. Malinowski, "Factor Analysis in Chemistry", 2nd edn., Wiley Interscience, New York, 1991.

[32] (a) M. Maeder and A. Zuberbühler, *Anal. Chem.*, **62**, 2220 (1990). A software package for global analysis based on Zuberbühler's methods is available commercially from Spectrum Software Associates, Chapel Hill, NC 27515-4494, USA, binstead@gibbs.oit.unc.edu. (b) K.-H. Müller and Th. Plesser, *Eur. Biophys. J.*, **19**, 231 (1991). (c) M. Carey, *EPA Newsletter*, **52**, 33 (1994).

[33] H. Gampp, M. Maeder, C. J. Meyer, and A. Zuberbühler, *Talanta*, **33**, 943 (1986). H. Gampp, M. Maeder, C. J. Meyer, and A. Zuberbühler, *Comments Inorg. Chem.*, **6**, 41 (1987).

[34] P. Bugnon, J.-C. Chottard, J.-L. Jestin, B. Jung, G. Laurenczy, M. Maeder, A. E. Merbach, and A. Zuberbühler, *Anal. Chim. Acta*, **298**, 193 (1994).

[35] J. Saltiel, J.-O. Choi, D. F. Sears, D. W. Eaker, F. B. Mallory, and C. W. Mallory, *J. Phys. Chem.*, **98**, 13612 (1994).